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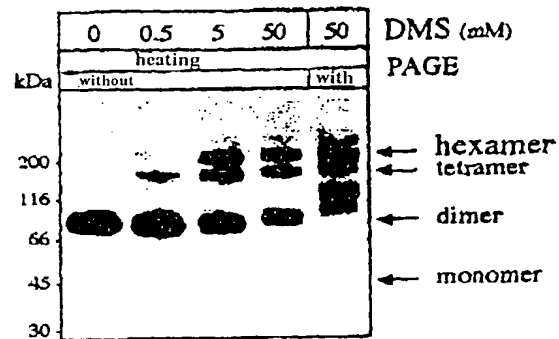


FIG. 1a

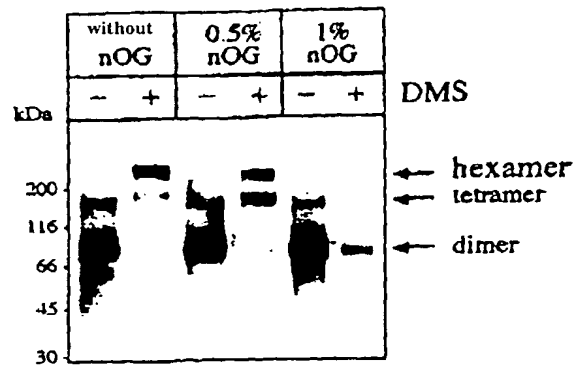


FIG. 1b

REPLACEMENT SHEET (RULE 26)

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SEQ ID N° 1 : NS1 protein of dengue virus serotype 1

```
1/1      31/11
atg agg agc gcg ccg ctt ccg atg acg tgc att gca gct ggc atg gct aca ccg tac cta
Met arg ser ala ser leu ser met thr cys ile ala val gly met val thr leu tyr leu
61/21    91/31
gga gtc acg gtt caa gcg gac ccg gga cgt gta atc aac tgg aag ggc aga gaa ctc aaa
gly val met val gln ala asp ser gly cys val ile asn trp lys gly arg glu leu lys
121/41   151/51
tgt gga agt ggc att ctt gtc att aat gaa gtc cac att tgg aca gag caa tac aaa ttc
cys gly ser gly ile phe val thr asn glu val his thr trp thr glu gln tyr lys phe
181/61   211/71
cag gcc gac tcc cca aaa aga ccg tca gca gcc att ggg aag gca tgg gag gag ggc gtc
gln ala asp ser pro lys arg leu ser ala ala ile gly lys ala trp glu glu gly val
241/81   271/91
tgt gga att cga tca gcc acg cgt ctt gag aac atc atg tgg aag caa ata tca aat gaa
cys gly ile arg ser ala thr arg leu glu asn ile met trp lys gln ile ser asn glu
301/101  331/111
ccg aac cac att cta ctt gaa aat gac atg aaa ccc aca gcg gct gta gga gat gct aat
leu asn his ile leu leu glu asn asp met lys phe thr val val val gly asp ala asn
361/121  391/131
gga att ccg gcc cag ggg aaa aaa atg att agg cca caa ccc atg gaa cac aaa tac tca
gly ile leu ala gln gly lys lys met ile arg pro gln pro met glu his lys tyr ser
421/141  451/151
tgg aaa agc tgg gga aaa gcc aag att ata gga gca gac aca cag aac acc acc ttc att
ccp lys ser trp gly lys ala lys ile ile gly ala asp thr gln asn thr thr phe ile
481/161  511/171
acc gac ggc cca gac att cca gaa tgc ccc gat gac caa aga gcg tgg aac att tgg gaa
ile asp gly pro asp thr pro glu cys pro asp asp gln arg ala trp asn ile trp glu
541/181  571/191
gtt gag gac tat ggg ttt gga att ttt acg aca aac aca tgg ccg aaa tgg cgt gac tcc
val glu asp tyr gly phe gly ile phe thr thr asn ile trp leu lys leu arg asp ser
601/201  631/211
cac acc caa acg tgt gac cac cgg cta atg cca gct gcc gtc aag gac agc aag gca gtc
tyr thr gln met cys asp his arg leu met ser ala ala val lys asp ser lys ala val
661/221  691/231
cat gct sac acg ggg tac tgg ata gaa agt gaa aag aac gag acc tgg aag cta gcg aga
his ala asp met gly tyr trp ile glu ser glu lys asn glu thr trp lys leu ala arg
721/241  751/251
gcc tcc tcc aca gaa gcc aag aca tgc att tgg ccg aaa tcc cac acc cca tgg agt aat
ala ser phe ile glu val lys thr cys ile trp pro lys ser his thr leu trp ser asn
781/261  811/271
gga gtt ccg gaa agt gaa atg ata att cca aag ata tat gga gga cca ata cct cag cac
gly val leu glu ser glu met ile ile pro lys ile tyr gly gly pro ile ser gln his
841/281  871/291
aat tac aga cca ggg tat ttc aca caa aca gca ggg cca tgg cac cta ggt aag tgg gaa
asn tyr arg pro gly tyr phe thr gln thr ala gly pro trp his leu gly lys leu glu
901/301  931/311
ttg gat tct gac ccg cgt gaa ggc acc aca gct gct gtc gat gaa cat tgt gga aat cga
leu asp phe asp leu cys glu gly thr thr val val val asp glu his cys gly asn arg
961/321  991/331
ggc cca tcc tcc aga att aca aca gtc aca gga aag ata atc cat gaa tgg cgt tgc aga
gly pro ser leu arg thr thr thr val thr gly lys ile ile his glu trp cys cys arg
1021/341 1051/351
tcc tgc acg cta ccc ccc cca cgc ttc aga gga gaa gac gga tgt tgg tat ggc acg gaa
ser cys thr leu pro pro leu arg phe arg gly glu asp gly cys trp tyr gly met glu
1081/361 1111/371
att aga cca gct aag gag aag gag gag aac cta gct agg tca atg gtc tcc gca taa
ile arg pro val lys glu lys glu glu asn leu val arg ser met val ser ala
```

FIG. 2

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I.D.	IHA				MAC	O.D. during NSI assay		
	D1	D2	D3	D4	ELISA	10 th	30 th	90 th
NOL 1	0	0	0	NR	NEG	0.24	0.16	0.13
NOL 2	10	40	10	NR	POS	0.13	0.10	0.09
NOT 1	0	0	0	NR	NEG	0.53	0.26	0.14
NOT 2	40	160	160	NR	POS	0.01	0.01	0.01
MONT 1	0	0	0	NR	NEG	1.02	0.67	0.44
MONT 2	320	40	20	NR	POS	0.03	0.02	0.02
BAIL 1	0	0	0	NR	NEG	1.20	0.83	0.64
BAIL 2	80	160	160	NR	POS	0.06	0.07	0.08
LEG 1	0	0	0	0	NEG	1.50	1.09	0.56
LEG 2	160	80	160	NR	POS	0.05	0.05	0.06
SGH 1	0	0	0	NR	NEG	0.76	0.54	0.26
SGH 2	80	640	160	NR	POS	0.03	0.02	0.03
DETT 1	0	0	0	NR	NEG	0.23	0.13	0.09
DETT 2	640	160	160	NR	POS	0.05	0.05	0.04
CON 1	0	0	0	0	NEG	0.23	0.10	0.10
CON 2	1280	1280	1280	1280	POS	0.12	0.07	0.03
BOLL 1	0	0	0	0	NEG	0.42	0.48	0.23
BOLL 2	1280	1280	1280	1280	POS	0.09	0.08	0.20
PORN 1	0	0	0	NR	NEG	0.54	0.54	0.44
PORN 2	1280	1280	1280	1280	POS	0.02	0.02	0.01
PAJ 1	0	0	0	0	NEG	1.71	1.27	0.73
PAJ 2	1280	1280	1280	1280	POS	0.07	0.07	0.06
PLAQ 1	10	20	20	NR	NEG	1.32	0.86	0.43
PLAQ 2	1280	1280	1280	1280	POS	0.28	0.18	0.18
GOH 1	10	40	20	NR	NEG	1.35	1.15	0.26
GOH 2	1280	1280	1280	1280	POS	0.06	0.09	0.14
BEAU 1	10	20	20	40	POS	1.01	0.76	0.53
BEAU 2	1280	1280	1280	1280	POS	0.07	0.08	0.07

Figure 3

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	ELISA NS <sub>1</sub> +		ELISA NS <sub>1</sub> -		% POSITIVITY FOR NS <sub>1</sub>
	MAC IgM -	MAC IgM +	MAC IgM -	MAC IgM +	
D <sub>0</sub>	2				-
D <sub>1</sub>	13		3		81.2
D <sub>2</sub>	8	1	6		64.3
D <sub>3</sub>	10	4	1		93
D <sub>4</sub>	4	6			100
D <sub>5</sub>	1	6			100
D <sub>6</sub>		4		1	80
D <sub>7</sub>		1		1	-
D <sub>8</sub>				1	-
D <sub>9</sub>		1		2	-
D <sub>11</sub> to D <sub>66</sub>				33	0

Figure 4

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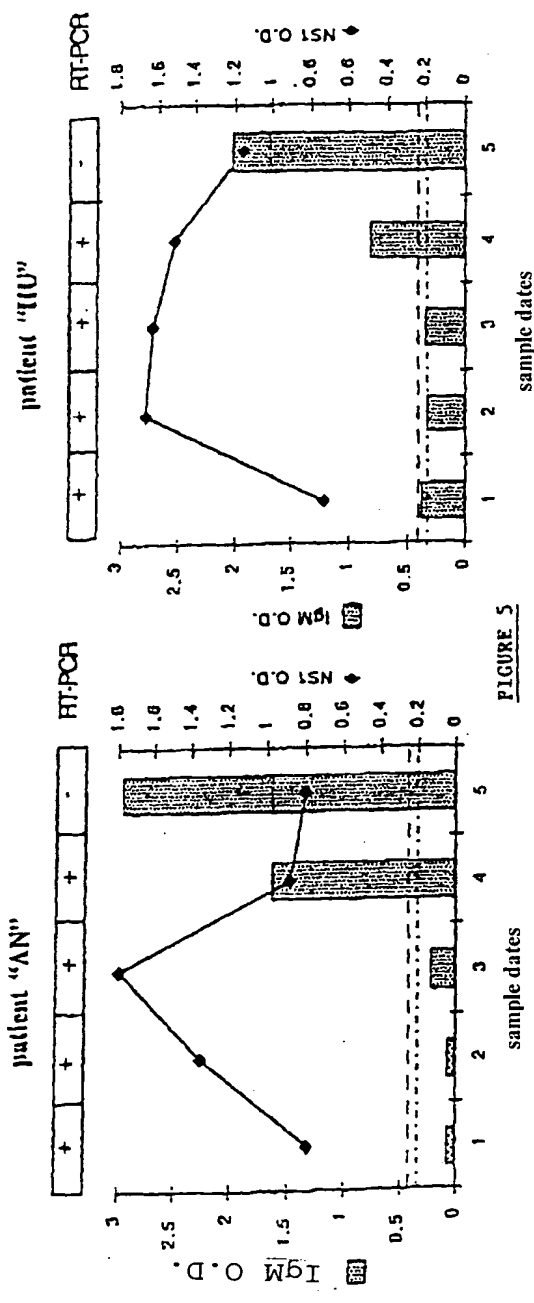
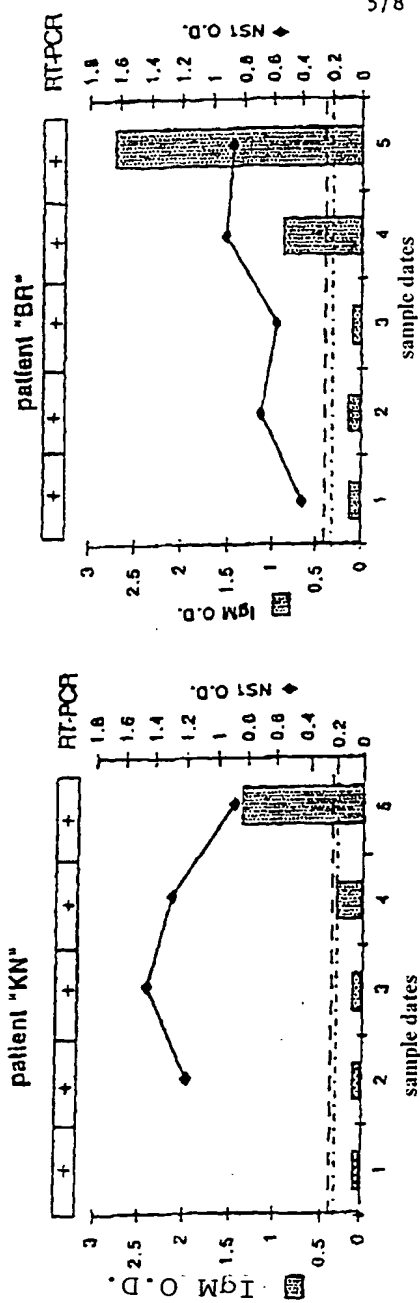
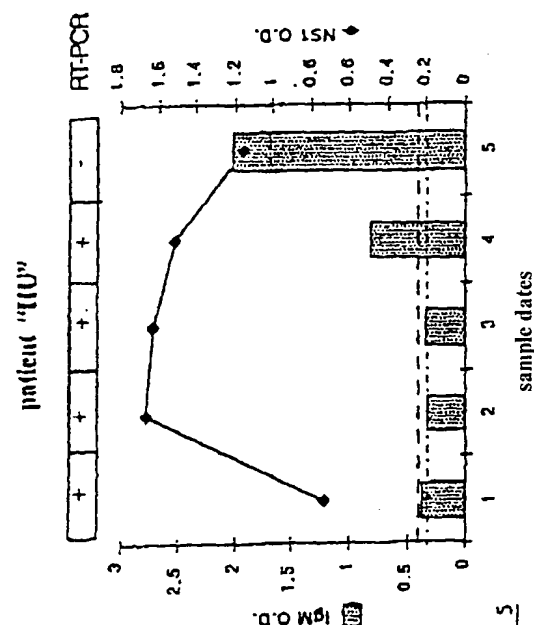
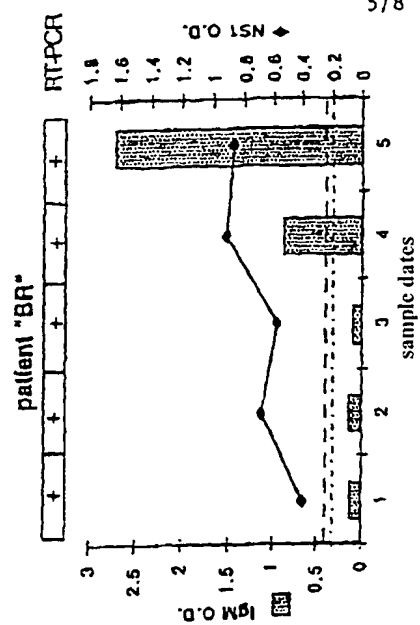


FIGURE 5



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F22

G18

REACTIVITY IN ELISA  
(DEN1 NSI)

purified soluble hexameric NSI

+

+

immunocaptured soluble  
hexameric NSI

+

+

REACTIVITY IN INDIRECT  
IMMUNOFLUORESCENCE

DEN1 - infected cells

+

+

DEN2 - infected cells

-

-

DEN3 - infected cells

-

+

DEN4 - infected cells

-

-

ISOTYPE

IgG1 K

IgG1 K

AFFINITY CONSTANT

 $2.7 \cdot 10^9 \text{ M}$  $3 \cdot 10^{11} \text{ M}$ 

EPITOPE

A

B

**Figure 6**

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patient no.	FD	IgM	O.D. with monoclonals			E.D. with polyclonals		
			10 <sup>th</sup>	30 <sup>th</sup>	90 <sup>th</sup>	10 <sup>th</sup>	30 <sup>th</sup>	90 <sup>th</sup>
314	1	-	0.33	0.22	0.10	0.11	0.07	0
231	3	-	0.80	0.68	0.33	0.41	0.22	0.05
292	3	-	0.84	0.74	0.43	0.47	0.33	0.16
304	3	+	1.23	0.92	0.59	0.66	0.41	0.22
371	3	+	1.10	1.10	0.81	0.68	0.49	0
88	4	-	1.24	1.27	1.19	1.13	0.95	0.35
106	5	+	1.28	1.25	1.26	0.95	0.86	0.16
383	13	+	0.04	0.04	0.04	0.09	0.07	0.01
222	29	+	0.01	0.01	0.02	0.07	0.06	0
267	50	+	0.01	0.01	0	0.06	0.05	0.03

FIGURE 7

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Serum No.	OD MAC IgM FJ	OD NS1 FJ	viral isolation
1	0.306	1.13	+
2	0.535	1.20	+
3	0.364	0.6	
4	0.578	0.65	
5	0.741	0.75	
6	0.968		
7	1.013		
8	1.101		
9	1.159		
10	1.278	0.61	
11	1.336		
12	1.448		
13	1.466		
14	1.501		
15	1.523	1.19	
16	1.587		
17	1.940		
18	2.109		

Figure 8